

Application No.: 10/562,089
Attorney Docket No.: 47675-066US0
First Applicant's Name: Catherine Lofton-Day
Application Filing Date: January 28, 2008
Date of Restriction Requirement: November 16, 2009
Date of Response: March 16, 2010
Examiner: Sarae L. Bausch

IN THE CLAIMS:

Applicants, pursuant to 37 C.F.R. § 1.121, submit the following amendments to the claims:

1. (Currently amended) A method for the detection of a colorectal cell proliferative disorder[[s]], comprising determining, in a genomic DNA sample of a subject, ~~at~~the CpG methylation status of two ~~or more of the~~ genes comprising ALX4[[,]] and TPEF, p16/INK4A, APC, ~~eaveolin-2, DAPK and TIMP3~~ and/or their regulatory sequences, and deducing, wherein based on said determined methylation status, the presence or absence a colorectal cell proliferative disorder or metastasis ~~is deduced~~.

2. (Cancelled)

3. (Cancelled)

4. (Currently amended) A method for the analysis of colorectal cell proliferative disorders, comprising determining the CpG methylation status of the gene ALX4 and/or its regulatory sequences, wherein based on said determined methylation status the presence or absence of a colorectal cell proliferative disorder or metastasis is deduced.

5. (Currently amended) ~~The method of claim 4~~A method for the analysis of colorectal cell proliferative disorders, further comprising determining the CpG methylation status of the gene ~~TPEF~~ALX4 and/or its regulatory sequences ~~and one or more of the genes selected from the group consisting TPEF, p16/INK4A, APC, caveolin-2, DAPK and TIMP3 and/or their regulatory sequences, and deducing, wherein~~ based on said determined methylation status, the presence or absence of a colorectal cell proliferative disorder or metastasis ~~is deduced~~.

6. (Withdrawn) A nucleic acid molecule consisting essentially of a sequence at least 18 bases in length according to a sequences selected from the group consisting of SEQ ID NOS:7, 8, 15 and 16.

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7. (Withdrawn-currently amended) An oligomer, in particular an oligonucleotide or peptide nucleic acid (PNA)-oligomer, for the detection of colon cell proliferative disorders, said oligomer consisting essentially of at least one base sequence having a length of at least ~~10~~20 contiguous nucleotides which ~~hybridises to or~~ is identical to a nucleic acid sequence selected from the group consisting of SEQ ID NOS:7, 8, 15 and 16 or to the complements thereof.

8. (Currently amended) The method of any one of claims 1, 4 and 5~~claim 1~~, comprising:
~~-obtaining, from a subject, a biological sample having subject genomic DNA;~~
~~-contacting the genomic DNA[[,]] or a fragment thereof, obtained from a subject, with one reagent or a plurality of reagents for distinguishing between methylated and non methylated CpG dinucleotide sequences within at least two~~one target sequences of the genomic DNA[[,]] or fragment thereof, wherein the target sequence comprises, or hybridizes under stringent conditions to, at least 16 contiguous nucleotides of a sequence ~~selected~~taken from the group consisting of SEQ ID NOS:7, 8, 9, 10, 15, 16, 17 and 18~~5 to SEQ ID NO:20 and SEQ ID NOS:48 to SEQ ID NO:59~~ said contiguous nucleotides comprising at least one CpG dinucleotide sequence; and
~~-determining, based at least in part on said distinguishing, the methylation state of at least one target CpG dinucleotide sequence, or an average, or a value reflecting an average methylation state of a plurality of target CpG dinucleotide sequences, whereby detecting, or detecting colon cell proliferative disorders~~ or metastasis is, at least in part, afforded.

9. (Original) The method of claim 8, wherein distinguishing between methylated and non methylated CpG dinucleotide sequences within the target sequence comprises converting unmethylated cytosine bases within the target sequence to uracil or to another base that is detectably dissimilar to cytosine in terms of hybridization properties.

10. (Original) The method of claim 8, wherein the biological sample is selected from the group consisting of cell lines, histological slides, biopsies, paraffin-embedded tissue, bodily fluids,

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stool, blood, and combinations thereof.

11. (Currently amended) The method of claim 8, wherein distinguishing between methylated and non methylated CpG dinucleotide sequences within the at least one target sequence comprises use of at least one nucleic acid molecule or peptide nucleic acid (PNA) molecule comprising, in each case a contiguous sequence at least 9 nucleotides in length that is complementary to, or hybridizes under moderately stringent or stringent conditions to a sequence selected from the group consisting of SEQ ID NOS: 7, 8, 9, 10, 15, 16, 17 and 18~~5 to SEQ ID NO: 20 and SEQ ID NOS: 48 to SEQ ID NO: 59~~, and complements thereof.

12. (Currently amended) The method of claim 8[[1]], comprising:

a) ~~obtaining, from a subject, a biological sample having subject genomic DNA;~~

[[b)]]extracting or otherwise isolating ~~the~~ genomic DNA from a subject biological sample having genomic DNA;

[[c)]]treating the isolated genomic DNA ~~of b)~~, or a fragment thereof, with one or more reagents to convert cytosine bases that are unmethylated in the 5-position thereof to uracil or to another base that is detectably dissimilar to cytosine in terms of hybridization properties;

[[d)]]contacting the treated genomic DNA, or the treated fragment thereof, with an amplification enzyme and at least two primers comprising, in each case a contiguous sequence of at least 9 nucleotides that is complementary to, or hybridizes under stringent conditions to a sequence selected from the group consisting of SEQ ID NOS: 7, 8, 9, 10, 15, 16, 17 and 18~~5 to SEQ ID NO: 20 and SEQ ID NOS: 48 to SEQ ID NO: 59~~, and complements thereof, wherein the treated genomic DNA or the fragment thereof is either amplified to produce at least one amplificate, or is not amplified; and

[[e)]]determining, based on a presence or absence of, or on a property of said amplificate, the methylation state of at least one CpG dinucleotide of ~~a sequence selected from the group~~

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consisting SEQ ID NOS: 2 and 3, ~~1 to SEQ ID NO: 4 and SEQ ID NOS: 45 to SEQ ID NO: 47~~ or an average, or a value reflecting an average methylation state of a plurality of CpG dinucleotides of a sequence selected from the groups consisting of SEQ ID NOS: 2 and 3 ~~1 to SEQ ID NO: 4 and SEQ ID NOS: 45 to SEQ ID NO: 47~~, wherein ~~whereby~~ at least one of detecting, or detecting and distinguishing between colon cell proliferative disorders is, ~~at least in part~~, afforded.

13. (Currently amended) The method of claim 12, wherein treating the genomic DNA, or the fragment thereof ~~in e)~~, comprises use of a reagent selected from the group consisting of bisulfite, hydrogen sulfite, and disulfite, ~~and combinations thereof~~.

14. (Currently amended) The method of claim 12, wherein contacting or amplifying ~~in d)~~ comprises use of at least one method selected from the group consisting of: use of a heat-resistant DNA polymerase as the amplification enzyme; use of a polymerase lacking 5'-3' exonuclease activity; use of a polymerase chain reaction (PCR); and generation of a ampificate nucleic acid molecule carrying a detectable labels; ~~and combinations thereof~~.

15. (Original) The method of claim 14, wherein said nucleic acid molecule or peptide nucleic acid molecule is in each case modified at the 5'-end thereof to preclude degradation by an enzyme having 5'-3' exonuclease activity.

16. (Original) The method of claim 14, wherein said nucleic acid molecule or peptide nucleic acid molecule is in each case lacking a 3' hydroxyl group.

17. (Original) The method of claim 16, wherein the amplification enzyme is a polymerase lacking 5'-3' exonuclease activity.

18. (Currently amended) The method of claim 12, wherein determining ~~in e)~~ comprises hybridization of at least one nucleic acid molecule or peptide nucleic acid molecule in each case comprising a contiguous sequence at least 9 nucleotides in length that is complementary to, or hybridizes under stringent conditions to a sequence selected from the group consisting of SEQ ID

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NOS:7, 8, 9, 10, 15, 16, 17 and 18, 5 to SEQ ID NO:20 and SEQ ID NOS:48 to SEQ ID NO:59and complements thereof.

19. (Original) The method of claim 18, further comprising extending at least one such hybridized nucleic acid molecule by at least one nucleotide base.

20. (Currently amended) The method of claim 12, wherein determining ~~in e)~~, comprises sequencing of the amplificate.

21. (Currently amended) The method of claim 12, wherein contacting or amplifying ~~in d)~~, comprises use of methylation-specific primers.

22. (Withdrawn-currently amended) A kit comprising a bisulfite reagent as well as at least one oligomer consisting essentially of at least one base sequence having a length of at least 10 contiguous nucleotides which hybridises to or is identical to a nucleic acid sequence[[s]] selected from the group consisting of SEQ ID NOS:7, 8, 9, 10, 15, 16, 17 and 18~~5 to SEQ ID NO:0 and SEQ ID NOS:48 to SEQ ID NO:59~~.

23. (Cancelled)

24. (New) The method of claim 8, wherein distinguishing between methylated and non methylated CpG dinucleotide sequences within the target sequence comprises the use of methylation sensitive restriction enzymes.